Supplementary Material (ESI) for Chemical Communications

Electronic supplementary information for:

Galactose Oxidase models: ^{19}F NMR as a powerful tool to study the solution chemistry of tripodal ligands in presence of copper(II).

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General: All chemicals were of reagent grade and used without purification. Microanalyses were performed by the Service Central d'Analyses du CNRS (Lyon, France).

298 K UV-vis spectra were recorded on a Perkin Elmer Lambda 2 spectrophotometer (1.000 cm path length quartz cell).

NMR spectra were recorded on a Bruker AM 300 (^{1}H at 300 Mhz, ^{13}C at 75 Mhz, ^{19}F at 282 MHz). Chemical shifts are given relative to tetramethylsilane (TMS). C_6F_6 was used as internal reference for ^{19}F NMR experiments (δ_{C6F6} = -162.17 ppm *vs.* $CFCl_3$).

Mass spectra were recorded on a Thermofunnigen (EI/DCI) or a Nermag R 101 C (FAB+) apparatus.

X-band EPR spectra were recorded on a BRUKER ESP 300E spectrometer equipped with a BRUKER nitrogen flow cryostat. Spectra were treated using the WINEPR software and simulated using the BRUKER SIMFONIA software.

Synthetic procedure:

6-fluoro-quinolin-ylmethyl)-pyridin-2-ylmethyl-amine: 6-fluoro-quinoline-2-carbaldehyde (1.0 g, 5.71 mmol) is dissolved in methanol (10 mL). Picolylamine (617 mg, 5.75 mmol in 2 mL methanol) is added dropwise during 30 min. The reaction mixture was stirred for 4 hours at room temperature. Sodium borohydride (216 mg, 5.71 mmol) is then added by small amounts, at 273 K during 30 min. After 3 h stirring, the organic layer was evaporated and HCl (2 M) is added until neutralization. The reaction mixture was extracted with CH_2Cl_2 (2×50 mL), washed with saturated NaCl, dried over Na_2SO_4 and evaporated. 6-fluoro-quinolin-ylmethyl)-pyridin-2-ylmethyl-amine was purified by silica gel column chromatography with CH_2Cl_2 : MeOH (20 : 1) as eluent. 6-fluoro-quinolin-ylmethyl)-pyridin-2-ylmethyl-amine (686 mg, 75%) was obtained as a red oil; ^{1}H NMR (300.12 MHz, $CDCl_3$, 298 K, TMS): δ = 3.46 (s, 1 H), 4.06 (s, 2 H), 4.17 (s, 2 H), 7.15 (dd, ^{3}J(H,H) = 7.7 Hz), 7.35-7.47 (m, 3 H), 7.50 (d, ^{3}J(H,H) = 8.5 Hz), 7.63 (td, 1 H, ^{3}J(H,H) = 7.7 Hz,^{4}J(H,H) = 1.8 Hz), 8.03 (d, ^{3}J(H,H) = 8.7 Hz, 1 H), 8.05 (d, ^{3}J(H,H) = 9.1 Hz, 1 H), 8.56 (d, ^{3}J(H,H) = 4.8 Hz, 1 H); ^{13}C NMR (75.465 MHz, $CDCl_3$, 298 K, TMS): δ = 55.1 (CH_2), 55.3 (CH_2), 110.8 (d, CH, ^{2}J(C,F) = 21.6 Hz), 119.8 (d, CH, ^{2}J(C,F) = 25.4 Hz), 121.6 (CH), 122.4 (CH), 122.8 (CH), 128.2 (d, Cq, ^{3}J(C,F) = 10.0 Hz), 131.8 (d, CH, ^{3}J(C,F) = 9.1 Hz), 136.2 (d, CH, ^{4}J(C,F) = 5.2 Hz), 136.8 (CH), 145.1 (Cq), 149.7 (CH), 159.5 (d, Cq, ^{4}J(C,F) = 2.8 Hz), 159.6 (Cq), 160.5 (d, Cq, ^{1}J(C,F) = 247.3 Hz) ; ^{19}F NMR (188.313 MHz, $CDCl_3$, 298 K, TMS): δ = 47.8 (td, 1F, ^{3}J(F,H) = 8.5 Hz,^{4}J(F,H) = 6.1 Hz); MS (DCI, NH_3 isobutane), m/z, 268 $(M+H)^+$.

2-*tert*-butyl-6-{[(6-fluoro-quinolin-2-ylmethyl)-pyridin-2-ylmethyl-amino]-methyl}-4-nitro-phenol: 3-*tert*-butyl-2-hydroxy-5-nitro-benzaldehyde (500 mg, 2.2 mmol, prepared according to F. Thomas et al., *Chem. Eur. J.*, **2003**, *9*, 3803), 6-fluoro-quinolin-ylmethyl)-pyridin-2-ylmethyl-amine (598 mg, 2.2 mmol) and 0.1 mL glacial acetic acid are dissolved in 50 mL methanol. Sodium cyanoborohydride (280 mg, 4.4 mmol) is added by small amounts, during 8 hours. After 12 h stirring, the reaction mixture is neutralized by HCl (2 M), extracted with CH_2Cl_2 (2×50 mL), washed with saturated NaCl, dried over Na_2SO_4 and evaporated. 2-tert-butyl-6-{[(6-fluoro-quinolin-2-ylmethyl)-pyridin-2-ylmethyl-amino]-methyl}-4-nitro-phenol was purified by silica gel column chromatography one time with CH_2Cl_2 : MeOH (60 : 1) as eluent and an other time with diethyl ether followed by diethyl ether : methanol (10 : 1). 2-*tert*-butyl-6-{[(6-fluoro-quinolin-2-ylmethyl)-pyridin-2-ylmethyl-amino]-methyl}-4-nitro-phenol (275 mg, 30 %) was obtained as a yellow oil; ^{1}H NMR (300.12 MHz, $CDCl_3$, 298 K, TMS): δ = 1.56 (s, 9 H), 3.94 (s, 4 H), 4.11 (s, 2 H), 7.16 (dd, 1 H, ^{3}J(H,H) = 7.4 Hz), 7.30-7.42 (m, 3 H), 7.51 (ddd, 1 H, ^{3}J(H,F) = 8.4 Hz, ^{3}J(H,H) = 8.3 Hz, ^{4}J(H,H) = 2.8 Hz), 7.60 (td, 1 H, ^{3}J(H,H) = 7.7 Hz, ^{4}J(H,H) = 1.8 Hz), 7.95 (d, 1 H, ^{4}J(H,H) = 2.8

Hz), 8.04 (d, 1 H, $^{3}J(H,H)$ = 8.6 Hz), 8.17 (d, 1 H, $^{4}J(H,H)$ = 2.8 Hz), 8.24 (dd, 1 H, $^{3}J(H,H)$ = 9.4 Hz, $^{4}J(H,F)$ = 5.4 Hz), 8.56 (d, $^{3}J(H,H)$ = 4.9 Hz); ^{13}C NMR (75.465 MHz, $CDCl_3$, 298 K, TMS): δ = 29.6 (CH_3, tBu), 35.6 (Cq), 57.3 (CH_2), 59.3 (CH_2), 59.7 (CH_2), 111.1 (d, CH, $^{2}J(C,F)$ = 21.5 Hz), 120.3 (d, CH, $^{2}J(C,F)$ = 25.3 Hz), 121.8 (CH), 122.8 (CH), 123.4 (CH), 123.5 (CH), 123.9 (Cq), 125.0 (CH), 128.3 (d, Cq, $^{3}J(C,F)$ = 10.1 Hz), 131.3 (d, CH, $^{3}J(C,F)$ = 9.0 Hz), 136.7 (d, CH, $^{4}J(C,F)$ = 5.5 Hz), 137.3 (CH), 138.1 (Cq), 139.5 (Cq), 144.7 (Cq), 149.2 (CH), 158.0 (d, Cq, $^{4}J(C,F)$ = 2.8 Hz), 158.2 (Cq), 160.8 (d, Cq, $^{1}J(C,F)$ = 248.3 Hz), 163.4 (Cq); ^{19}F NMR (282.395 MHz, $CDCl_3$, 298 K, TMS): δ = 48.5 (td, 1F, $^{3}J(F,H)$ = 8.4 Hz, $^{4}J(F,H)$ = 5.1 Hz); MS (DCI, NH_3 isobutane), m/z, 475 $(M+H)^+$. Anal. Calcd for $C_{27}H_{27}FN_4O_3$: C, 68.34; H, 5.74; N, 11.81 %. Found: C, 67.95; H, 5.78; N, 11.78 %.

(**1**)(ClO_4^-): 2-*tert*-butyl-6-{[(6-fluoro-quinolin-2-ylmethyl)-pyridin-2-ylmethyl-amino]-methyl}-4-nitro-phenol (300 mg, 0.633 mmol) was treated by 1 equivalent of NEt_3 in CH_3CN (10 mL) prior to addition of 1 eq of $Cu(ClO_4)_2$•6 H_2O (3 ml CH_3CN solution, 234 mg, 0.633 mmol). (**1**)(ClO_4^-) was recrystallized by slow diffusion of Et_2O into a CH_3CN solution. Single brown crystals of (**1**)(ClO_4^-) were formed and collected by filtration (342 mg, 80 %). ESI MS, m/z, 536 (M - CH_3CN - ClO_4^+). Anal. Calcd for $C_{29}H_{29}ClCuFN_5O_7$: C, 51.41; H, 4.31; N, 10.34. Found: C, 51.26; H, 4.35; N, 10.40 %. UV-Vis (λ_{max} [nm] [ε / $M^{-1}.cm^{-1}$]): 510 [910], 654 [260]. EPR (9.41 GHz, CH_3CN, 100 K): broad signal at g ≈ 2.

(**1H**)(ClO_4^-)$_2$: 2-*tert*-butyl-6-{[(6-fluoro-quinolin-2-ylmethyl)-pyridin-2-ylmethyl-amino]-methyl}-4-nitro-phenol (300 mg, 0.633 mmol) was dissolved in CH_3CN (10 mL) prior to addition of 1 eq of $Cu(ClO_4)_2$•6 H_2O (3 ml CH_3CN solution, 234 mg, 0.633 mmol). The volume is reduced to 1 ml, and dry acetonitrile (10 mL) is added. The operation is repeated three times, until the solution turns from green to blue. (**1H**)(ClO_4^-)$_2$ was recrystallized by slow diffusion of iPr_2O (on $CaCl_2$) into a CH_3CN solution. Blue crystals of (**1H**)(ClO_4^-)$_2$ were formed and collected by filtration (200 mg, 40 %). ESI MS, m/z, 536 (M - H - CH_3CN - ClO_4^+). Anal. Calcd for $C_{29}H_{29}Cl_2CuFN_5O_{11}$: C, 44.77; H, 3.89; N, 9.00. Found: C, 44.70; H, 3.89; N, 8.90 %. UV-Vis (λ_{max} [nm] [ε / $M^{-1}.cm^{-1}$]): 630 [120]. EPR (9.41 GHz, CH_3CN, 100 K): $g_{xx} = g_{yy}$ = 2.065, g_{zz} =2.241, A_{xx} = 1.0 mT, A_{yy} = 1.5 mT, A_{zz} = 17.2 mT.

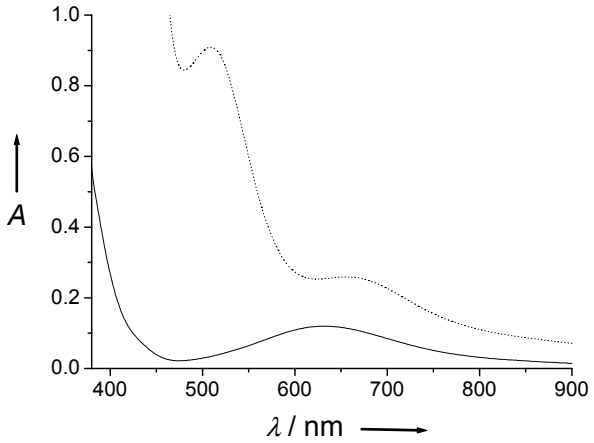

Fig S1: UV-Vis spectra of 1 mM CH_3CN solutions of **1H** (solid lines) and **1** (dotted lines). T = 298 K, l = 1.000 cm.

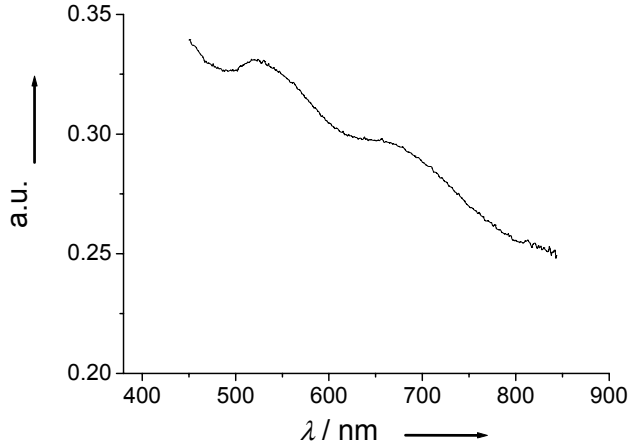

Fig S2: Transmittance spectrum of a crystalline powder of **1**. T = 298 K.

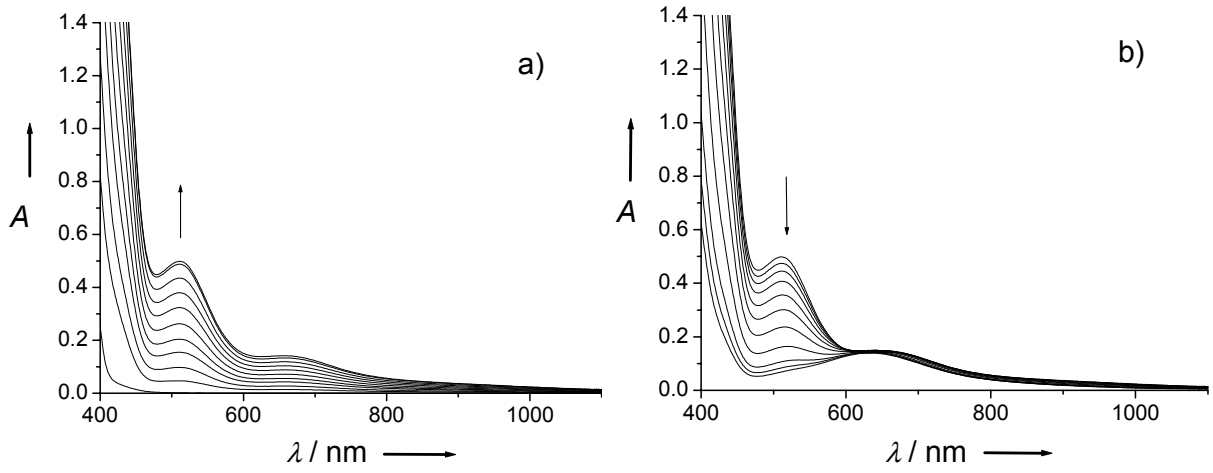

Fig S3: Titration of HLq^{NO2} (0.98 mM) by copper(II) perchlorate: a) 0 to 0.5 copper molar equivalent and b) 0.5 to 1 copper molar equivalent. Arrows indicate spectral changes upon addition of copper; spectra were recorded in CH_3CN at 298 K. l = 1.000 cm.

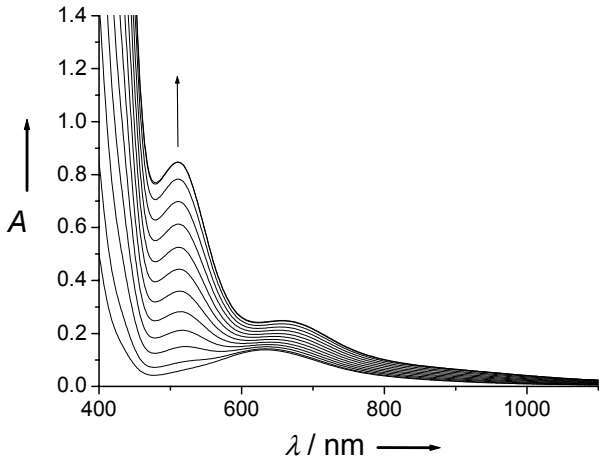

Fig S4: Titration of **1H** (0.97 mM) by 0 to 1 molar equivalent of NEt_3. Arrows indicate spectral changes upon addition of base; spectra recorded in CH_3CN at 298 K. l = 1.000 cm.

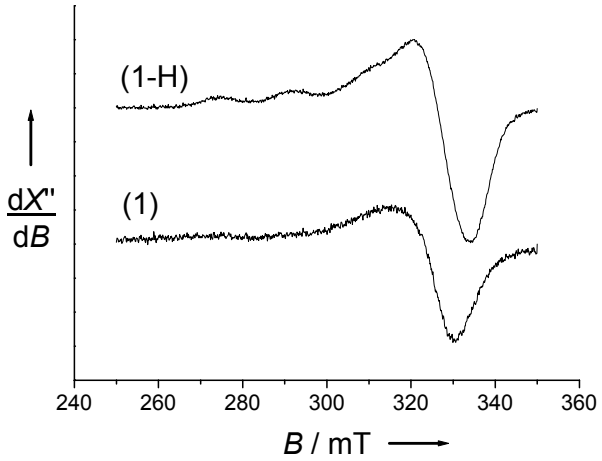

Fig S5: X-Band EPR spectra (normalized) of 1 mM CH_3CN solutions of **1H** and **1**. Microwave freq. 9.419 GHz, power: 20 mW; Mod. Freq. 100 kHz, amp. 0.197 mT (**1H**) and 0.0987 mT (**1**); T = 100 K.

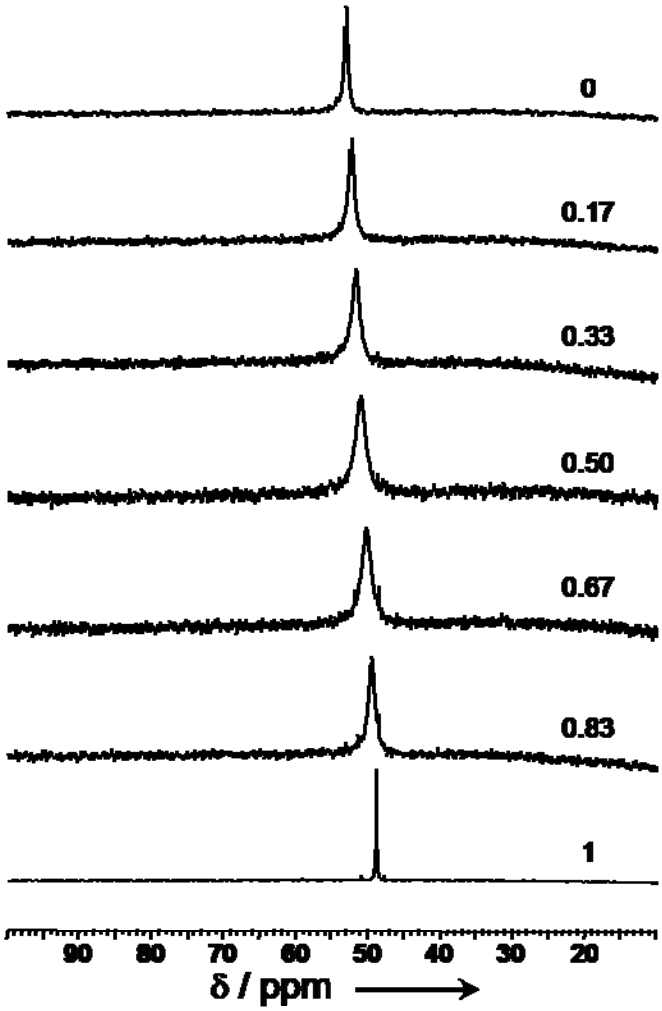

Fig S6: Titration of **1H** (60 mM) by NEt_3; ^{19}F NMR spectra were recorded in (CD_3CN : CH_3CN) (1 : 4) at 293 K. The numbers correspond to the molar equivalents of base added. The sharp peak at 48 ppm corresponds to an unidentified impurity, accounting for less than 1% of the total ^{19}F signals. *Intensities are normalized*.

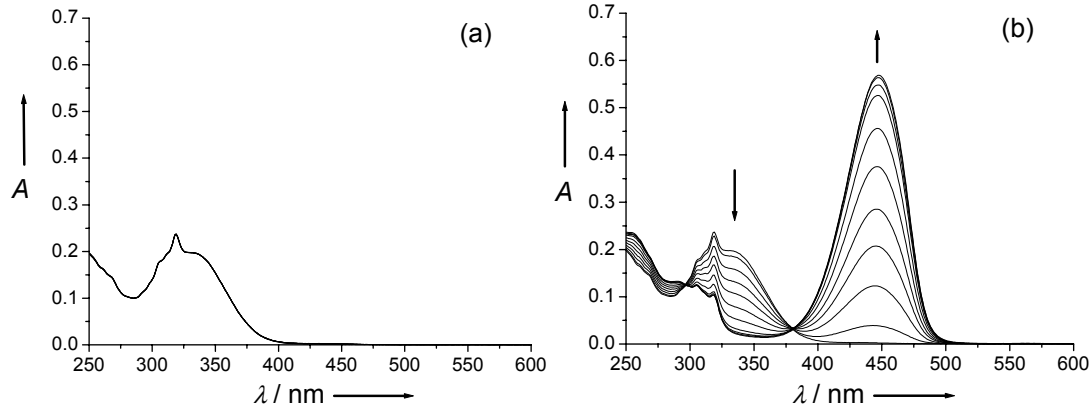

Fig S7: Titration of HLq^{NO2} (1.86×10^{-5} M) by: a) 0 to 1 copper molar equivalent of NEt_3 (no spectral changes could be observed) and b) 0 to 1 copper molar equivalent of nBu_4NOH, showing that only OH^- deprotonates the nitrophenol of HLq^{NO2}. Arrows indicate spectral changes upon addition of base; spectra were recorded in CH_3CN at 298 K. l = 1.000 cm.